



United States
Department of
Agriculture

Grain Inspection,
Packers and Stockyards
Administration

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TO: FGIS POLICY BULLETIN BOARD

FROM: David Orr, Director /S/
Field Management Division

SUBJECT: Revision to the StarLink Testing and Certification Procedures

ORIGINATING OFFICE: Policies and Procedures Branch

1. BACKGROUND

This memorandum transmits revisions to the official StarLink testing and certification procedures. The Technical Services Division (TSD) has recently verified the performance of the Trait✓ Bt9 Lateral Flow Test Kit (Strategic Diagnostics Incorporated), and the QuickStix™ Test Kit (EnviroLogix Incorporated), to detect the presence of the Cry9C protein in corn at a level of 0.125 percent (1 kernel in 800).

The increased sensitivity of the test kits will necessitate several changes to FGIS Directive 9181.1, "Testing for StarLink™ Corn", dated 1-26-01. These changes include step-by-step testing instructions for the SDI and Envirologix tests and a new certification statement.

Additionally, FGIS is redefining the meaning of a "subsample" for qualitative StarLink™ testing using the SDI and Envirologix tests. A "subsample" is defined as a sample portion of up to 800 kernels (approximately 230 grams) in size that is used to perform StarLink™ testing. Simplified, each lateral flow test strip used for a test is considered as a subsample. For example, at a detection level of 1 in 2400 kernels, use 3 subsamples of 800 kernels (3 test strips capable of detecting at the 0.125 sensitivity level) or 6 subsamples of 400 kernels (6 test strips capable of detecting at the 0.25 sensitivity level) for analysis.

2. TEST KITS

All Envirologix test kits can detect the presence of the Cry9C protein in corn at a level of 0.125 percent (1 kernel in 800). Please follow the enclosed step-by-step instructions for testing.

SDI test kits (Trait✓Bt9) identified with part number 7000034 are restricted to testing at the 1 in 400 kernel level. Test kits identified with part number 7000003 are approved for testing at a level of 0.125 percent (1 kernel in 800). Please follow the enclosed step-by-step instructions for testing.

NOTE: SDI Test Kits (part number 7000003) do not include buffer solution.

3. TRAIT✓Bt9 TESTING PROCEDURES (part number 7000034)

a. Processing the Sample.

- (1) Place each sub-sample of 115 grams (\pm 5 grams), approximately 400 kernels, in a clean, dry "Mason" jar or similar container used for blending purposes. **(Container must hold a minimum of 16 fluid ounces.)**
- (2) Attach the container and **clean, dry cutting blades** to the mixing device (food processor or blender). Place a plastic shield over the container (for protection) in case the container breaks during sample processing.
- (3) Grind the sub-sample on high speed until all whole kernels are broken (approximately 30 seconds).

Note: An alternative method to grind the sub-sample is permitted provided the particle size is comparable to the blender procedure and processes are in place to prevent cross-sample contamination.

- (4) Remove the sub-sample container from the mixing device and add 143 to 145 milliliters (ml) of water.

NOTE: If the analytical portion size was adjusted to approximate 400 kernels, adjust the water volume using the formula:

Sample weight (grams) X 1.25 = Water volume (ml)

- (5) Place a lid on the container and shake to thoroughly wet all corn particles (approximately 10 - 20 seconds). Let the slurry settle for 15 - 30 seconds. The sub-sample will have a thick consistency but should contain some free liquid.
- (6) Transfer 0.5 ml of the free liquid from the container and place in a 1.5-ml sample tube using a transfer pipette provided with the test kit.

Note: Some corn material will be transferred with the free liquid in this step.

- (7) Add 5 drops of the Trait✓ Sample Buffer into the sample tube.

Caution: Failure to add 5 drops of buffer to the tube may cause false positive results.

- (8) Close the tube and shake for approximately 10 seconds.
- (9) Place 1 Trait✓ Bt9 Test Strip into the sample tube. Allow the strip to remain in the test tube for a full 10 minutes. If a control line (top of the strip) does not develop the test is invalid. Reading the strip prior to 10 minutes could result in false negative results.

Caution: The top of the liquid in the sample tube should not be higher than the top of the arrows on the test strip when it is in the sample tubes.

b. Interpreting the Lateral Flow Test Strip.

Check the result window frequently after inserting the strip. At least one line, the Control Line, should always develop approximately 1 centimeter down from the Reservoir Pad. A red line in this position indicates that the device is functioning properly. A red line appearing below the Control Line is the Test Line and indicates a positive result.

NOTE: Development of the Control Line serves only to indicate that the device has functioned properly. Discard any test strip that does not develop a Control Line and re-test the sample using another strip.

4. TRAIT✓Bt9 TESTING PROCEDURES (part number 7000003)

a. Processing the Sample.

- (1) Place each sub-sample 230 grams (\pm 5 grams), approximately 800 kernels, in a clean, dry "Mason" jar or similar container used for blending purposes. **(Container must hold a minimum of 32 fluid ounces.)**
- (2) Attach the container and **clean, dry cutting blades** to the mixing device (food processor or blender). Place a plastic shield over the container (for protection) in case the container breaks during sample processing.
- (3) Grind the sub-sample on high speed until all whole kernels are broken (approximately 30 seconds).

Note: An alternative method to grind the sub-sample is permitted provided the particle size is comparable to the blender procedure and processes are in place to prevent cross-sample contamination.

- (4) Remove the sub-sample container from the mixing device and add 285 to 287 milliliters (ml) of water.

NOTE: If the analytical portion size was adjusted to approximate 800 kernels, adjust the water volume using the formula:

Sample weight (grams) X 1.25 = Water volume (ml)

- (5) Place a lid on the container and shake to thoroughly wet all corn particles (approximately 10 - 20 seconds). Let the slurry settle for 15 - 30 seconds. The sub-sample will have a thick consistency but should contain some free liquid.
- (6) Transfer 0.5 ml of the free liquid from the container and place in a 1.5-ml sample tube using a transfer pipette provided with the test kit.

Note: Some corn material will be transferred with the free liquid in this step.

- (7) Place 1 Trait✓ Bt9 Test Strip into the sample tube. Allow the strip to remain in the test tube for a full 10 minutes. If a control line (top of the strip) does not develop the test is invalid. Reading the strip prior to 10 minutes could result in false negative results.

Caution: The top of the liquid in the sample tube should not be higher than the top of the arrows on the test strip when it is in the sample tubes.

b. Interpreting the Lateral Flow Test Strip.

Check the result window frequently after inserting the strip. At least one line, the Control Line, should always develop approximately 1 centimeter down from the Reservoir Pad. A red line in this position indicates that the device is functioning properly. A red line appearing below the Control Line is the Test Line and indicates a positive result.

NOTE: Development of the Control Line serves only to indicate that the device has functioned properly. Discard any test strip that does not develop a Control Line and re-test the sample using another strip.

c. Alternate Test Levels.

Testing can be performed at different levels (e.g., 1 in 400 kernels, 1 in 600 kernels) by reducing the amount of sample and water volume. Use the following table to determine the appropriate sample size and water volume for alternate test levels.

Test Level	Sample Size (Kernels)	Sample Size (Grams)	Water Volume
1 in 600	600 kernels	171 (\pm 5 grams)	214 - 216 ml
1 in 400	400 kernels	115 (\pm 5 grams)	143 - 145 ml
1 in 200	200 kernels	57 (\pm 5 grams)	71 - 73 ml

5. QUICKSTIX™ TESTING PROCEDURES

a. Processing the Sample.

- (1) Place each sub-sample 230 grams (\pm 5 grams), approximately 800 kernels, in a clean, dry "Mason" jar or similar container used for blending purposes. **(Container must hold a minimum of 32 fluid ounces.)**
- (2) Attach the container and **clean, dry cutting blades** to the mixing device (food processor or blender). Place a plastic shield over the container (for protection) in case the container breaks during sample processing.
- (3) Grind the sub-sample on high speed until all whole kernels are broken (approximately 30 seconds).

Note: An alternative method to grind the sub-sample is permitted provided the particle size is comparable to the blender procedure and processes are in place to prevent cross-sample contamination.

- (4) Remove the sub-sample container from the mixing device and add 365 to 367 milliliters (ml) of **distilled or deionized water**.

NOTE: If the analytical portion size was adjusted to approximate 800 kernels, adjust the water volume using the formula:

Sample weight (grams) X 1.6 = Water volume (ml)

- (5) Place a lid on the container and shake to thoroughly wet all corn particles (approximately 10 - 20 seconds). Let the slurry settle for 15 - 30 seconds. The sub-sample will have a thick consistency but should contain some free liquid.
- (6) Using the transfer pipette provided with the test kit, draw up enough liquid portion to fill the long narrow tip of the pipet up to the line at the top of the pipet bulb. Avoid pulling up particles of corn.
- (7) Dispense the extract into a reaction tube.
- (8) Place 1 QuickStix™ Test Strip into the sample tube. After inserting the strip into the reaction tube you will observe liquid travelling up the membrane strip toward the absorbent pad at the top of the strip. Allow the strip to remain in the test tube for a full 10 minutes. If a control line (top of the strip) does not develop the test is invalid. Reading the strip prior to 10 minutes could result in false negative results.

b. Interpreting the Lateral Flow Test Strip.

At least one line, the Control Line, should always develop. A red line in this position indicates that the device is functioning properly. A red line appearing below the Control Line is the Test Line and indicates a positive result.

NOTE: Development of the Control Line serves only to indicate that the device has functioned properly. Discard any test strip that does not develop a Control Line and re-test the sample using another strip.

c. Alternate Test Levels.

Testing can be performed at different levels (e.g., 1 in 400 kernels, 1 in 600 kernels) by reducing the amount of sample and water volume. Use the following table to determine the appropriate sample size and water volume for alternate test levels.

Test Level	Sample Size (Kernels)	Sample Size (Grams)	Water Volume
1 in 600	600 kernels	171 (\pm 5 grams)	274 - 276 ml
1 in 400	400 kernels	115 (\pm 5 grams)	182 - 184 ml
1 in 200	200 kernels	57 (\pm 5 grams)	91 - 93 ml

6. CERTIFICATION

To accurately certify StarLink test results we are adding information to the certification statement to identify the test results in terms of testing levels (e.g., 800 kernels). Use the following statement to certify all test results:

"(Negative/Positive) StarLink test result based on (number) kernels ([number] sub-samples)."

For example: "Negative StarLink test result based on 800 kernels (1 subsample)."

7. QUESTIONS

Please direct any questions regarding this policy to the Policies and Procedures Branch. Please pass this information to the agencies and grain industry in your area.